United States Environmental Protection Agency **Office of Research and Development**

National Exposure Research Laboratory
Research Abstract

Government Performance Results Act (GPRA) Goal 8
Annual Performance Measure 66

Significant Research Findings:

Report on the Effectiveness of Genetic Marker Methods as Indicators of Condition of Eastern U.S. Streams

Scientific Problem and Policy Issues

ORD's Ecological Research Strategy calls for the "development of a set of indicators for estuarine, stream and lake systems that can be interpreted relative to the status and changes in fundamental ecological and hydrologic processes that influence and constrain the integrity and sustainability of these systems." Existing ecological indicators fall short of this goal, and the EPA is working to develop new, innovative methods that fill key information gaps. Measures of genetic diversity within species have been suggested as potentially powerful indicators of ecological condition. Genetic diversity is a key component of biodiversity. It is shaped by environmental forces and helps determine future population risks, thus genetic diversity measures are potentially both prospective and retrospective indicators of the condition of populations. While measures of genetic diversity within species have great potential, they have not been thoroughly analyzed as tools for regional monitoring and assessment programs. This report summarizes work by the EPA to gauge the effectiveness of measures of genetic diversity within stream fish species as one component of a regional monitoring and assessment program.

Research Approach

The main objective of this study is to evaluate the potential of recent DNA marker technologies to contribute to regional assessments of ecological condition. Several key subobjectives are considered:

(1) Can molecular population genetic methods be used to delineate biogeographic boundaries between stream fish populations? These biogeographic boundaries define the fundamental units of ecological organization and are key to interpretation of environmental assessment data.

(2) Does the amount of molecular genetic diversity within stream fish populations vary among streams? If so, does this measure of genetic diversity correlate with key measures of environmental condition?

(3) What is the most efficient design for a regional genetic monitoring

program? Design questions include choice of the most appropriate molecular marker technology, sampling issues, and laboratory and analytical resource requirements.

These objectives are evaluated based on results of pilot studies of genetic diversity in two stream fish species common to Eastern U.S. streams. Sample sites were probabilistically chosen as part of Regional Environmental Monitoring and Assessment Program (REMAP) and Environmental Monitoring and Assessment Program (EMAP) assessments. Samples were analyzed using a number of different genetic methods, including RAPD, AFLP, and mitochondrial DNA sequencing.

Results and Impact

Results of the pilot studies indicate that incorporation of molecular genetic information into regional ecological assessments could have great value. Genetic differences between populations of two common minnow species (central stoneroller and creek chub) were shown to be very large. Sample sites could be clustered into groups such that all sites within a group were genetically and demographically related, but different groups were highly independent. Biogeographic boundaries could be defined based on these groupings that appeared to be similar to, although not identical to, watersheds or U.S. Geological Survey hydrologic units.

Levels of molecular genetic diversity within sites differed significantly across the sites, regardless of the type of molecular marker used to assess genetic diversity. Levels of genetic diversity appear to correlate with key environmental factors, although the significance of this correlation may vary with species. Genetic diversity of creek chubs in the Allegheny Plateau Ecoregion is strongly associated with levels of nitrogen, phosphorous, organic carbon, and pH.

While results are largely comparable for different molecular markers, the results suggest that RAPD technology is not cost-effective relative to other options. At present, it is recommended that a dedicated molecular marker laboratory be enlisted to aid in regional genetic assessments, but this will change as the cost and expertise needed for DNA-level analyses diminishes. Theoretical considerations suggest that genetic data will be particularly informative in the context of temporal monitoring, and regional assessments should be designed with this capability in mind.

This research supports ORD's research to improve the scientific foundation of ecological risk assessment under the Government Performance and Results Act (GPRA) Goal 8.1.1 (Sound Science, Improved Understanding of Environmental Risk and Greater Innovation to Address Environmental Problems).

These diagnostic indicator development studies address the Goal 8.1 Multiyear Plan Programmatic Long-Term Goal for Ecological Protection:

"Condition Research": "The States and Tribes assess the condition of all

their waters in a scientifically-defensible and representative fashion that allows aggregation and assessment of trends at multiple scales."

2006 Interim Goal: "Method to effectively monitor trends in environmental condition with known confidence are available to EPA, the States and Tribes."

2006 Annual Performance Goal (APG): "Use of probability sampling for detecting trends in ecosystem condition established."

The work is of primary importance to EPA's EMAP and REMAP programs and similar State and Tribal environmental assessment programs.

Research Collaboration and Research Products

This research was conducted primarily by a team of National Exposure Research Laboratory (NERL) Ecological Exposure Research Division (EERD) staff scientists. Significant collaborators include the Ohio EPA and EPA Region 5 Office. Contractor assistance in collecting samples and performing molecular marker analyses was provided by Sobran, Inc. Preliminary research findings were presented at the scientific conferences for the Society for Environmental Toxicology and Chemistry (2000, 2001) and the American Society of Ichthyology and Herpetology (2002). The following publications resulted from this research effort:

Silbiger, R.N., Leonard, A.C., Dimsoski, P., Fore, S., Guttman, S.I., Roth A.C., Gordon, D.A., Wessendarp, T.,Toth, G.P. and Smith, M.K.. Use of Molecular Markers to Study the Effects of Environmental Impacts on Genetic Diversity in Brown Bullhead (*Ameirus nebulosus*) Populations. *Environmental Toxicology and Chemistry* 20(11):2580-2587, 2001.

Silbiger, R.N., Christ, S.A., Leonard, A.C., Garg, M., Dawes, S., Dimsoski, P., McCormick, F., Wessendarp, T., Gordon, D.A., Roth, A.C., Smith, M.K., Toth, G.P. Preliminary studies on the population genetics of the central stoneroller (*Campostoma anomalum*) from the Great Miami River Basin, Ohio. *Environmental Monitoring and Assessment* 51: 481-495, 1998.

Leonard, A.C., Franson, S.E., Hertzberg, V.S., Smith, M.K., Toth, G.P. Hypothesis testing with the similarity index. *Molecular Ecology* 8:2105-2114, 1999.

Christ, S.A., Silbiger, R.N., Garg, M., Franson, S.E., Toth, G.P. Quality assurance considerations for use of the Fluorimager SI and FragmeNTanalysis software. *Electrophoresis* 21: 874-888, 2000.

Future Research

Our research results to date indicate that measures of genetic diversity are informative for assessment of ecological condition because they help define ecological units and appear to be responsive to environmental stresses. However, measures of genetic diversity may have still greater importance as measures of population vulnerability. This is because the capacity for populations to respond (adapt) to environmental change is proportional to the amount of genetic diversity within the populations. Planned research focuses on experimental determination of the importance of genetic diversity for buffering aquatic populations against environmental variability and predicting population vulnerability.

A second research focus is to evaluate the benefits of integrating genetic diversity measures, landscape indicators and population modeling tools into a single ecological assessment. The research is premised on the idea that genetic and demographic changes in population are linked and both should be tied to landscape-level changes.

Finally, ongoing research is aimed at evaluating the utility of long-term genetic monitoring of fish populations as an indicator of trends in the condition of aquatic ecosystems. This effort is in collaboration with the Department of Fisheries and Oceans, Canada, and uses a DNA archive that goes back 25 years for fish populations that have been experimentally exposed to a variety of stressors.

Contacts for Additional Information

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